SHOCK, Vol. 29, No. 6, pp. 748–753, 2008

IS SURVIVAL TIME AFTER HEMORRHAGE A HERITABLE, QUANTITATIVE TRAIT?: AN INITIAL ASSESSMENT

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Received 18 Jul 2007; first review completed 3 Aug 2007; accepted in final form 29 Aug 2007

ABSTRACT—Enhancing survival to hemorrhage of both civilian and military patients is a major emphasis for trauma research. Previous observations in humans and outbred rats show differential survival to similar levels of hemorrhage. In an initial attempt to determine potential genetic components of such differential outcomes, survival time after a controlled hemorrhage was measured in 15 inbred strains of rats. Anesthetized rats were catheterized, and approximately 24 h later, 55% of the calculated blood volume was removed during a 26-min period from conscious unrestrained animals. Rats were observed for a maximum of 6 h. Survival time was 7.7-fold longer in the longest-lived strain (Brown Norway/Medical College of Wisconsin; 306 ± 36 min; mean ± SEM) than in the shortest-lived strain (DA; 40 ± 5 min; $P \leq 0.01$). Mean survival times for the remaining inbred strains ranged from 273 ± 44 to 49 ± 4 min (Dahl-Salt Sensitive > Brown Norway > Munich Wistar Fromter > Dahl-Salt Resistant > Copenhagen > Noble > Spontaneous-hypertensive > Lewis > BDIX > Fawn Hooded Hypertensive > FISCHER 344 > Black agouti > PVG). The variance in the hazard of death attributable to different strains was estimated to be 1.22 log-hazard units, corresponding to a heritability of approximately 48%. Graded and divergent survival times to hemorrhage in inbred rat strains are remarkable and suggest multiple genetic components for this characteristic. However, this interpretation of differential responses to hemorrhage may be confounded by potential strain-associated differences related to the surgical preparation. Identification of inbred strains divergent in survival time to hemorrhage provides the opportunity for future use of these strains to identify genes associated with this complex response.

KEYWORDS—Inbred rats, genetic determinants, hemorrhagic shock, differential survival

INTRODUCTION

Historically (1) and currently (2, 3), approximately 50% of battlefield deaths occur due to hemorrhage. In the civilian sector, hemorrhage is the second leading cause of traumatic death (4–6). Additionally, unintentional injury (57% of which involve trauma) is the primary cause of death for all individuals younger than 45 years (7).

Physiological differences between patients who eventually die and those who survive have been documented after high-risk surgery or trauma involving significant hemorrhage (8–10). Similarly, in studies with outbred rats, comparisons between survivors and nonsurvivors to the same degree of controlled hemorrhage have described characteristics that differentiate the two groups (11). After acute hemorrhage, multiple body systems are activated in attempts to maintain homeostasis, especially to maintain perfusion of the brain and heart (12). Various physiological (11, 13), immunological (14, 15), and hormonal (12, 16) measures reflect this activation and provide clues to mechanisms associated with enhanced differential survival to hemorrhage. However, such measures are often taken at different time points after hemorrhage or in different tissues, thereby making clear conclusions as to their relevance in determining outcome difficult.

The complexity of the response to hemorrhage, and the multitude of factors potentially regulating this response, suggests that survival time after hemorrhage is a complex trait with many interacting genetic and environmental determinants. One might further speculate that survival time after hemorrhage represents a quantitative trait, that is, a complex trait that demonstrates continuous variation (17). This possibility further suggests an alternative genetic approach to discovering mechanisms that distinguish survivors from nonsurvivors. If survival time to hemorrhage is indeed regulated by genes, then identification of these genes should provide not only an understanding of variant response mechanisms but also the means to identify approaches to intervene in the pathophysiology of hypovolemia and improve survival in hemorrhage victims.

As an initial attempt to identify genes associated with survival time after hemorrhage, we tested the hypothesis that survival time after controlled hemorrhage is a heritable quantitative trait by measuring this phenotype in multiple strains of inbred rats.

MATERIALS AND METHODS

Animals

All rats were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. This study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Tex. Animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, revised 1996).

Charles River Laboratories (Wilmington, Mass) supplied the following inbred rat strains: BDIX (body weight at surgery, 331 ± 9 g, mean ± SEM), Brown Norway (BN; 259 ± 6 g), Brown Norway Medical College of Wisconsin (BN/Mcwi;

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This work was supported in part by funds associated with the Surviving Blood Loss Program of the Defense Advanced Research Projects Agency.
DOI: 10.1097/SHK.0b013e31815cfe30

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 JUN 2008	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Is survival time after hemorrhage a heritable, quantitative trait?: an initial assessment	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Klemcke H. G., Baer D. G., Pankratz V. S., Cox A., Cortez D. S., Garrett M. R., Joe B., Ryan K. L.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**6**	

276 ± 4 grams), Dahl Salt-Sensitive (S; 341 ± 16 g), Fawn Hooded Hypertensive (FHH; 361 ± 7 g), Noble (317 ± 10 g), and Outbred Sprague-Dawley (Outbred; 448 ± 8 g). Harlan (Indianapolis, Ind) supplied the following inbred rat strains: Black agouti (ACI; 230 ± 4 g), Copenhagen 2331 (COP; 250 ± 3 g), DA/OlaHsd (DA; 258 ± 6 g), FISCHER 344 (F344; 283 ± 8 g), Lewis (Lew; 302 ± 6 g), Munich Wistar Fromter (MWF; 276 ± 5 g), PVG/OlaHsd (PVG; 261 ± 4 g), Spontaneously Hypertensive (SHR; 323 ± 4 g), and Dahl Salt-Resistant (R; 316 ± 8 g). All rats were males that were shipped at approximately 10 weeks of age and were held for an 18- to 24-day acclimation period before experimentation. Rats were maintained individually in plastic cages (27.3 × 48.9 × 27.3 cm) at 19°C to 23°C, with lights on from 0600 to 1800 h, and food (Harlan Global Teklad 2018; Madison, Wis) and water constantly available. Relative humidity averaged 55% to 65% during each 24-h period. Rats were randomly assigned to day of surgery, order of surgery on each day, and order of hemorrhage on each day.

Surgical procedures

All surgical procedures were conducted under aseptic conditions. Before surgery, body weight and weight of food and water provided to the rat for the next 24 h were recorded. Rats were anesthetized with 2% to 5% isoflurane (Forane; Baxter Healthcare Corporation, Deerfield, Ill) in 100% oxygen. A catheter consisting of polyethylene (PE)50 tubing with a PE10 tip (Clay Adams, Parsipany, NJ) was inserted into the left common carotid artery and exteriorized in the dorsal neck region. The catheter was pretreated with tridodecylmethylammonium chloride–heparin (Polysciences, Inc., Warrington, Pa) before placement. A small blood sample was obtained after surgery using a heparinized capillary tube (Clear Crit; Separation Technologies, Altamonte Springs, Fla) and processed for hematocrit measures using a HemataSTAT (Separation Technologies). The catheter was then filled with 100 μL of sterile glycerol that did not contain an anticoagulant and sealed with a sterile stainless steel wire plug (1 cm × 0.635 mm). Rats were injected with buprenorphine (2.5 μg/100 g body weight, s.c.) and with 10 mL of 0.9% saline (s.c.) to provide analgesia and hydration during recovery.

Hemorrhage procedures

Approximately 24 h later, the rats and the remaining food and water were weighed. Rats were restrained briefly using a plastic DecapiCone (Braintree Scientific, Braintree, Mass) to attach a tubing extension to the PE50 tubing protruding from the dorsal neck. The rat was released into a transparent standard rat cage that allowed for easy observation of the rat throughout the hemorrhage and 6-h observation period. The extension tubing was then connected to a transducer (Deltrand IV; Utah Medical Products, Midvale, Utah) that in turn was connected to a Differential DC Amplifier and Signal Conditioner (Ectron Corp., San Diego, Calif). Blood pressure was measured and recorded at 5-s intervals in the freely moving rat. Baseline measures were recorded for 5 min before the transducer was disconnected, and the rat was subjected to hemorrhage through the same catheter. The rat's blood volume was calculated using the stable body weight just before surgery and the figure of 5.83 mL/100 g of body weight, which was an average blood volume derived from previous investigations (18–21). Fifty-five percent of the blood volume was withdrawn according to the following schedule: 25% of the blood volume to be removed was withdrawn at a constant rate during the first 4 min, whereas the remaining 75% of the blood volume to be removed was withdrawn at a constant rate during the next 22 min. Blood was removed manually using 1-mL syringes and close adherence to a timer such that rates of blood withdrawal among rats were as constant as possible. The second 0.5-mL blood sample and the penultimate 0.5-mL blood sample removed during hemorrhage were analyzed for hematocrit using the ABX Pentra 120 (ABX Diagnostics, Irvin, Calif). After hemorrhage, 1 mL of sterile saline (37°C) without anticoagulant was used to flush the catheter and extension. When rats displayed agonal breathing, cessation of breathing, or reached 6 h postinitiation of hemorrhage, they were euthanized with an intravascular injection of sodium pentobarbital (15 mg/l00 g body weight). To avoid potential influences of endogenous circadian rhythms, all rats were hemorrhaged between 0700 and 1200 h. Air temperature in the vicinity of the rat was maintained at approximately 25°C throughout the hemorrhage and subsequent observational period using a lamp.

Statistics

Data were analyzed using the Statistical Analysis System package (SAS, Cary, NC) and S-Plus (Insightful, Seattle, Wash). Surgical and bleeding-associated measures were analyzed using a single-way ANOVA (PROC ANOVA). Differences among individual means were examined using the *a posteriori* Student-Newman-Keuls test. Correlation analyses were conducted using PROC CORR of the SAS system. When appropriate, hematocrit data were analyzed using a repeated-measures procedure associated with the PROC MIXED program of SAS. Multiple mean comparisons using *t* tests were adjusted via control of the false discovery rate (22). All data were tested for homogeneity of variance (Levene's test) and normality of distribution (PROC Univariate Normal with associated Kolmogorov-Smirnov test). Data were transformed where necessary to meet assumptions of ANOVA. Many rat strains had rats that survived the complete 6 h and were euthanized. The true survival time of these rats is unknown, and such data are said to be "censored." Hence, these survival data with censored observations were analyzed using either PROC LIFETEST or PROC PHREG, with potential covariates detailed previously. PROC LIFETEST with associated Kaplan-Meier procedure for estimating survivor functions and log-rank test for determining differences among survivor functions were used to compare all inbred rat strains without covariates. PROC PHREG, which incorporates Cox regression and its associated proportional hazards model (23), was used with covariates. All covariates considered were initially included in the overall model, but then, through an iterative process, covariates that were found not to be significant were dropped from the model. Similarly, interactions between each significant covariate and rat strains were tested and retained in the final model if found to be significant. Validity of the proportional hazard assumption was examined by testing the interaction of the covariates with time (23). Probabilities of survival curve comparisons were adjusted for multiple comparisons via controlling the false discovery rate (22). Differences in percent survival were determined using PROC FREQ and associated chi-square test. Heritability of survival times was assessed by estimating a per-strain random effect (24). A second measure of heritability was obtained through the estimate of a pseudo-R-squared value from the partial likelihoods corresponding to the Cox proportional hazards regression model (25). Most data are presented as arithmetic mean ± SEM. However, because 9 of the 15 rat strains studied had censored data, survival times and associated SEM are underestimates due to the censored data (23).

RESULTS

Mean survival times varied over an approximately 8-fold range among strains (Fig. 1). DA rats had the shortest survival time (34 ± 10 min), whereas BN/Mcwi had the longest survival time (306 ± 36 min). Percent survival varied from 0 to 82 ($P < 0.001$; Fig. 1). Kaplan-Meier survival graphs of representative strains are presented that indicate the divergent responses throughout the 6-h observational period (Fig. 2). Use of the Cox proportional hazard model readily disclosed multiple differences among strains (Table 1).

Duration of surgery (52.3 ± 0.8 min; mean ± SEM for rats of all strains; $P = 0.3$), time interval between surgery and

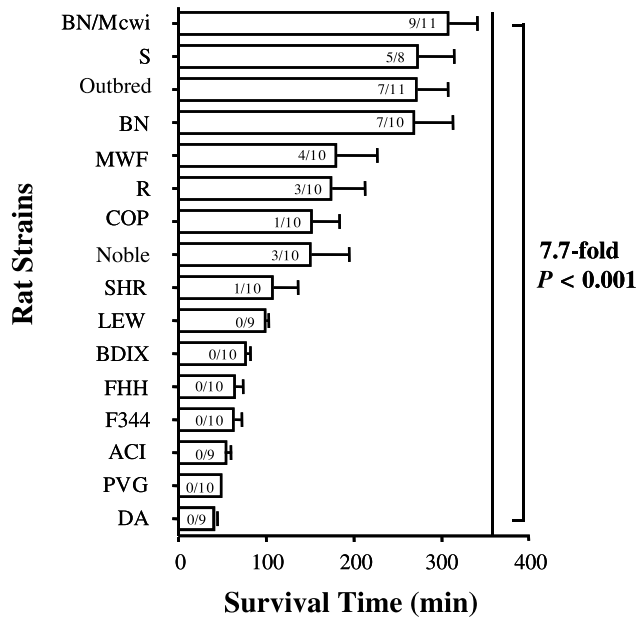

FIG. 1. **Survival time in inbred rat strains to a controlled hemorrhage (55% of body weight)**. Values within bars indicate the number of rats that lived / total number of rats for that strain. Bars represent the mean ± SEM. The vertical dashed line represents 360 min, the maximum observation period, at which rats were euthanized if still alive (censored data). Overall result of strain comparisons by PROC LIFETEST and associated log-rank test is presented.

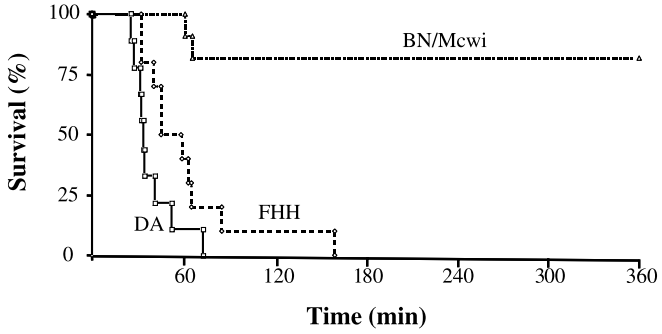

FIG. 2. **Kaplan-Meier survival curves for three representative inbred rat strains**. Rat strains are BN/Mcwi (n = 11), FHH (n = 10), and DA (n = 9).

hemorrhage (23.97 ± 0.18 h; $P = 0.89$), time period of restraint before hemorrhage (5.0 ± 0.2 min; $P = 0.73$), and measured percent hemorrhage volume based on the body weight at surgery (54.9% ± 0.1%; $P = 0.41$) did not differ among strains. These variables represented potential nongenetic factors that can influence survival time to the controlled hemorrhage. Indeed, when used as covariates, some were significant confounders in various strain comparisons using the Cox proportional hazard model and enhanced the accuracy of comparisons (Table 1).

Additional variables (Table 2) that can potentially influence survival to the hemorrhage showed strain-dependent differences ($P < 0.0001$). The percent of blood volume removed (calculated using the weight present just before hemorrhage; PVH) was greater than that calculated based on the body weight at surgery for each strain (57.1 ± 0.1 vs. 54.9 ± 0.1; PVH vs. percent of blood volume at surgery for all rats; $P < 0.001$) but did not differ among strains (Table 2). Hematocrit differed among rat strains on the day of surgery ($P < 0.001$; data not shown) and in blood samples taken at the beginning and end of the hemorrhage (Table 2). The changes in hematocrit during the bleed were not different among strains ($P = 0.8$; Table 2).

The variability in the log-hazard of death attributable to the several strains was estimated to be 1.22 units. This corresponded to a per-strain risk of death that commonly varied by 3-fold or more. This degree of per-strain variability corresponded to an estimate of heritability among all rat strains that was equal to 47.6%.

DISCUSSION

The current study is the first to demonstrate large differences in survival time to controlled hemorrhage among inbred rat strains. The continuous variation measured strongly supports the hypothesis that survival time to hemorrhage is a quantitative trait regulated by multiple genes. Furthermore, heritability estimates demonstrated that 48% of the variation in this complex phenotypic trait was attributable to additive genetic components specific to the different rat strains.

With an objective of identifying genes essential for enhanced survival to hemorrhage, the choice of animal models was critical for this initial work. Rats were chosen because they provided (1) a large number of inbred rat strains; (2) an animal that can be reproducibly catheterized and bled 24 h later; (3) a sufficient volume of blood in which multiple varied measures can be made; (4) a vast database of relevant physiological data, especially cardiovascular information (17, 26, 27); and (5) a rapidly accruing genetic database (28, 29). Furthermore, it was important to use an animal model that was not influenced by the potentially confounding effects of anesthetics and analgesics because such factors can affect responses to hemorrhage in a strain-dependent manner (30, 31) and thereby hinder our ability to identify genes primarily associated with the hemorrhage response itself. Because we chose to allow a 24-h period after surgery for recovery from isoflurane (anesthetic) and buprenorphine (analgesic) (32–34), alterations in food and water consumption and body weight during this period became an integral part of the hemorrhage model. Our data indicate that, indeed, the weight loss after surgery differed among rat strains. Consequently, the calculated percent of blood volume removed—if one uses the altered body weight present at hemorrhage—increased from the planned 55%. Even so, the percent of total blood volume removed using this calculation did not differ among rat strains. Our decision to use the stable body weight present before surgery in calculating hemorrhage volume was based on the fact that it represented a normal body composition unaltered in an unknown manner by the stressor of surgery; therefore, this decision seems justified due to the similarity of results obtained with the two methods of calculation. However, it must be remembered that the blood volume removed for each strain was calculated based on an average blood volume as a percentage of body weight obtained from outbred rats (18–21). The possibility exists that blood volume might vary in a strain-dependent manner. If this possibility is indeed true, then this would be construed as one of the genetic variables affecting survival time to hemorrhage. This hypothesis awaits further exploration and, if true, must be taken into account in future work.

TABLE 1. **Comparisons of survival time to controlled hemorrhage for selected inbred rat strains**

Comparison	Adjusted *P*
All rat strains	0.0005
BN/Mcwi vs. BN	0.71
BN/Mcwi vs. MWF	0.07
BN/Mcwi vs. Noble	0.01
BN/Mcwi vs. SHR	0.009
BN/Mcwi vs. LEW	0.008
BN/Mcwi vs. FHH	0.005
BN/Mcwi vs. DA	0.003
S vs. FHH	0.006
DA vs. FHH	0.08
MWF vs. SHR	0.33
LEW vs. DA	0.006

Comparisons were calculated via Cox regression and associated proportional hazards model (PROC PHREG). For individual strain comparisons, significance levels are those after controlling for any significant covariates (duration of surgery, time between surgery and hemorrhage, period for which the rat was restrained before hemorrhage, and blood volume removed as a percentage of the rats' body weight). Probability levels have been adjusted for multiple comparisons (22).

TABLE 2. **Independent, strain-related variables potentially related to survival time to hemorrhage**

Strain	n	Water consumed (g) postsurgery per 100 g BW	Food consumed (g) postsurgery per 100 g BW	Weight loss between surgery and bleed as percent BW at surgery	Percent of blood volume removed based on BW at hemorrhage	Initial HCT*	Final HCT*	Change HCT	Initial MAP
BN/Mcwi	11	$4.7 \pm 0.5^{a,b,c,d}$	$5.3 \pm 0.2^{a,b}$	1.5 ± 0.6^{a}	56.8 ± 0.4	$37.1 \pm 0.6^{e,f}$	$28.3 \pm 0.9^{d,e}$	8.6 ± 1.3	116 ± 2^{c}
S	8	$3.3 \pm 0.7^{c,d}$	$5.2 \pm 0.4^{a,b}$	$3.6 \pm 0.8^{a,b,c}$	56.5 ± 0.6	$39.3 \pm 0.5^{c,d,e}$	28.2 ± 1.3^{a}	11.0 ± 2.1	$123 \pm 4^{b,c}$
Outbred	11	$4.9 \pm 0.5^{a,b,c,d}$	$5.5 \pm 0.3^{a,b}$	$3.3 \pm 0.6^{a,b,c}$	56.8 ± 0.2	$40.9 \pm 0.9^{a,b,c,d}$	$29.8 \pm 1.8^{b,c,d,e}$	11.1 ± 1.9	115 ± 3^{c}
BN	10	$6.0 \pm 1.0^{a,b,c}$	$5.5 \pm 0.8^{a,b}$	$3.6 \pm 0.7^{a,b,c}$	56.5 ± 0.6	$41.8 \pm 1.3^{a,b,c}$	$30.9 \pm 1.1^{b,c,d,e}$	10.7 ± 1.0	$120 \pm 3^{b,c}$
MWF	10	$4.5 \pm 0.5^{a,b,c,d}$	$5.4 \pm 0.3^{a,b}$	$5.1 \pm 0.8^{a,b,c}$	57.5 ± 0.4	$37.7 \pm 1.8^{d,e,f}$	26.7 ± 2.2^{e}	11.3 ± 3.6	$138 \pm 3^{b,c}$
R	10	$7.6 \pm 0.9^{a,b}$	7.8 ± 0.5^{a}	$2.2 \pm 0.7^{a,b}$	56.0 ± 0.4	$42.5 \pm 0.9^{a,b,c}$	$30.2 \pm 1.2^{b,c,d,e}$	11.8 ± 0.8	$124 \pm 2^{b,c}$
COP	10	$4.7 \pm 0.8^{a,b,c,d}$	$5.1 \pm 0.4^{a,b}$	$3.9 \pm 1.0^{a,b,c}$	57.1 ± 0.4	$36.9 \pm 1.7^{e,f}$	$29.7 \pm 1.1^{b,c,d,e}$	8.9 ± 1.0	$121 \pm 3^{b,c}$
Noble	10	$8.5 \pm 1.1^{a,b}$	$5.8 \pm 0.7^{a,b}$	$2.7 \pm 1.0^{a,b,c}$	56.7 ± 0.6	44.0 ± 1.0^{a}	35.8 ± 1.3^{a}	8.7 ± 0.9	$136 \pm 6^{b,c}$
SHR	10	$5.9 \pm 0.7^{a,b,c}$	$5.3 \pm 0.5^{a,b}$	$4.5 \pm 0.8^{a,b,c}$	57.4 ± 0.7	$43.3 \pm 0.4^{a,b}$	$33.6 \pm 0.6^{a,b}$	9.7 ± 0.6	173 ± 6^{a}
LEW	9	2.5 ± 0.6^{d}	4.3 ± 0.7^{b}	$5.0 \pm 0.7^{a,b,c}$	57.2 ± 1.1	$40.0 \pm 2.6^{b,c,d,e}$	$31.6 \pm 1.5^{a,b,c,d}$	7.3 ± 3.2	95 ± 9^{d}
BDIX	10	$3.4 \pm 0.4^{b,c,d}$	4.5 ± 0.3^{b}	$3.1 \pm 0.6^{a,b,c}$	56.9 ± 0.3	$43.2 \pm 0.8^{a,b}$	$31.4 \pm 2.4^{b,c,d}$	11.6 ± 1.7	117 ± 2^{c}
FHH	10	$6.0 \pm 1.0^{a,b,c}$	4.5 ± 0.4^{b}	6.4 ± 0.5^{c}	58.0 ± 0.5	$39.4 \pm 0.7^{c,d,e}$	$31.1 \pm 1.5^{b,c,d}$	8.3 ± 1.7	$137 \pm 5^{b,c}$
F344	10	$2.9 \pm 0.4^{c,d}$	4.4 ± 0.4^{b}	$5.7 \pm 0.9^{b,c}$	57.8 ± 0.4	$42.0 \pm 2.1^{a,b,c}$	$33.0 \pm 0.7^{a,b,c}$	9.0 ± 2.3	$128 \pm 3^{b,c}$
ACI	9	$3.6 \pm 0.8^{c,d}$	4.5 ± 0.5^{b}	$3.1 \pm 0.9^{a,b,c}$	56.9 ± 0.5	35.1 ± 2.1^{f}	$30.6 \pm 1.1^{b,c,d,e}$	4.5 ± 2.5	116 ± 5^{c}
PVG	10	$6.2 \pm 0.9^{a,b,c}$	$5.5 \pm 0.3^{a,b}$	$5.9 \pm 1.0^{b,c}$	58.3 ± 0.5	$41.7 \pm 1.0^{a,b,c}$	$32.8 \pm 1.0^{a,b,c}$	8.9 ± 1.6	$126 \pm 3^{b,c}$
DA	9	9.8 ± 1.8^{a}	2.7 ± 0.4^{c}	$4.7 \pm 1.0^{a,b,c}$	57.3 ± 0.2	$39.0 \pm 1.3^{c,d,e}$	$28.4 \pm 1.2^{c,d,e}$	10.5 ± 1.0	144 ± 4^{b}
P from ANOVA		<0.0001	<0.0001	<0.0001	0.15	<0.0001	<0.0001	0.80	<0.0001

*The second 0.5-mL blood sample and the penultimate 0.5-mL blood sample removed during hemorrhage were analyzed for HCT using the ABX Pentra 120. Rat strains are arranged according to mean survival time (longest to shortest) after a 55% controlled hemorrhage.
Overall results of strain comparisons by analysis of variance (ANOVA) for the various factors are presented. Data represent the arithmetic mean ± SEM. Means that do not share a superscript (a, b, c, d, e, f) are significantly different ($P < 0.05$).
BW indicates body weight; HCT, hematocrit.

A related concern is potential strain-dependent differences in nutrition and hydration due to altered food and water intake subsequent to surgery (35). Because of this concern, a 10-mL injection of saline was provided immediately after surgery in an attempt to ensure appropriate hydration. One homeostatic response to hemorrhage involves the movement of interstitial fluid into the intravascular space (36). Historically, hematocrit has been used as a measure of the restoration of plasma volume after hemorrhage (37). Hematocrit values after hemorrhage may be influenced by nutritional and hydration status because rats with reduced food and water intake demonstrate smaller reductions of hematocrit than control rats, suggesting a decreased ability to translocate fluid into the intravascular space (38). In our current work, although baseline and final values for hematocrit differed among strains, there were no differences in the changes in hematocrit occurring during hemorrhage. These data would therefore suggest that attempts at restitution of blood volume in the different rat strains were not compromised by differences in food and water intake after surgery, at least during the period of hemorrhage.

Waiting for 24 h after surgery to initiate hemorrhage was considered a sufficient delay to obviate any confounding influences of isoflurane and buprenorphine on the responses to hemorrhage (32, 33, 39, 40). However, a third confounding factor is the recently demonstrated ability of isoflurane to reduce adverse effects of cardiac ischemia up to 72 h after exposure to 2 h of isoflurane (41, 42). Although isoflurane exposure in the current study was less than 1 h, the potential exists that isoflurane exposure during the surgery can influence the overall response to whole-body ischemia in a strain-dependent manner.

Although our study provides the first evidence for a contribution of genetic components to physiological responses to the total body ischemia of hemorrhage, previous studies have indicated differences among inbred strains in challenges relevant to traumatic injury. For example, inbred rat strains differ in their survival to burn injury (43), whereas inbred mouse strains differ in their survival to burn, mechanical, or radiation injury (44). Interestingly, the variability in the ability to survive burn injury studies (BN > LEW > ACI = F344) (43) seems to be similar to the variability in the ability to survive controlled hemorrhage (this study) in the rat strains common to both. Very recent data suggest that there are also genetic predispositions in human patients that are associated with early mortality following trauma (45).

Use of inbred strains has become a relatively common procedure for identifying and dissecting complex genetic traits (17, 27, 46, 47). Inbred strains are those that have been brother-sister mated for at least 20 generations and which are predicted to be 98.6% homozygous (48). Genetic variability among inbred rats of the same strain is very low but measurable (49); hence, phenotypic variability among rats of the same inbred strain is attributed primarily to environmental factors, whereas between-strain variability represents genetic

contributions (46). Although phenotypic differences among inbred strains are by themselves an indication that the observed character is under genetic control (30), the heritability calculations provide an additional quantification of this observation, suggesting that 48% of the differences in strain-specific survival times may be directly attributable to additive genetic effects (24). However, it should be remembered that an inherent challenge in this type of genetic dissection is that a gene's additive effect is dependent on the genetic background in which it is contained (50, 51).

The current study allows for selecting strains that can be applied for further mapping studies to arrive at the causal variants that dictate the phenotype of survival after hemorrhage. Such variants may exist as single nucleotide polymorphism (SNPs) or groups of SNPs in haplotypes. Identification of these causal SNPs or haplotypes that exist as variants between the two strains chosen for mapping studies is facilitated only through the process of linkage and/or mapping studies that can be further expedited with SNP analysis of the recombinant rats if and when a dense collection of rat SNP data becomes available. To expedite the identification of the specific underlying causative SNPs and/or other sequence variations such as deletions or insertions, it is possible to make use of consomic rat strains wherein single chromosomes are transferred from one inbred rat strain to another (27). Because such consomic rat strains are present for BN/Mcwi and FHH, use of these consomic rat strains will be made in subsequent studies.

The inbred strains chosen for our study reflect a mixture of considerations, including (1) known physiological characteristics, (2) historical use in hemorrhage and/or ischemia-related research, and (3) usefulness for future research models. For example, BN and S rats were tested because *in vitro* heart preparations from these strains have demonstrated relative resistance (BN) or sensitivity (S) to myocardial ischemia (52). Brown Norway, S, and FHH rats were also tested because they have been used as founder strains in the generation of multiple consomic rat breeds (27) that can be readily used in future searches for quantitative trait loci and genes associated with this survival time. DA, PVG, R, F344, ACI, LEW, and COP were previously tested for their aerobic running capacity (53). These strains were assessed in the current study because this capacity is in part a function of cardiovascular fitness, which can also be important to surviving hemorrhage. Finally, ACI (54, 55), LEW (56–58), PVG (56, 59), and SHR (60, 61) rats have been routinely used in various ischemia-related studies. Choice of strains was therefore made in consideration of the known body of literature that can help assist interpretation of data collected in future studies. The choice of strains tested in the current study was much less important than the demonstration that significant differences exist among these strains.

What are the physiological mechanisms that account for such differences in survival time to hemorrhage? The response to hemorrhage includes at least two major mechanisms: (1) reflex compensations by the cardiovascular system to maintain vital organ perfusion and (2) tissue responses to ischemia (11). We speculate that strains that are more sensitive to hemorrhage do not have an adequate initial cardiovascular response, whereas strains that survive have both an appropriate cardiovascular response and tissues that are more resistant to ischemia. For cardiovascular compensation, Barbato et al. (53) have demonstrated 1.8-fold differences in cardiac performance in isolated hearts from a variety of rat strains; however, those strains demonstrating the highest levels of cardiac performance (DA and PVG) were found to be the least resistant to hemorrhage in our study. These investigators later found that DA rats had higher levels of resting sympathetic tone than COP rats (62), suggesting that a diminished cardiovascular compensatory reserve may contribute to the susceptibility of DA rats to meet the hypovolemic challenge of hemorrhage. Inbred rat strains also differ in their susceptibility to cardiac (52), renal (56), and cerebral (63) ischemia; however, the rapid death of many strains argues against tissue ischemia being the primary mechanism. Although these data are suggestive of differences in the cardiovascular and tissue responses to hemorrhage, determination of the varying genetically determined mechanisms by which inbred rat strains respond to this challenge requires further investigation.

Taken together, data presented herein provide strong evidence that the ability to survive hemorrhage is a heritable quantitative trait. Given our current understanding of cellular, tissue, organ, and organism level regulation of compensatory mechanisms, it is likely that this trait is under the influence of a large number of genes. The significance of the current study is that it provides information directing us to an alternative, complementary course of investigation for answering our previously posed question concerning physiological mechanisms that account for differences in survival time to hemorrhage. When one makes an observation of physiological differences, one must at once ask regarding their cause. A course directed at identifying underlying genetic differences will address this question and provide tools to enhance and/or screen for genetic factors beneficial for survival to hemorrhage.

ACKNOWLEDGMENTS

The authors thank SGT Jason Bliss, Nahir Miranda, and JingJing Wang for technical assistance, and Dr. Bijan Kheirabadi for reviewing this article.

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